Journal of Phytology 2011, 3(9): 33-36
ISSN: 2075-6240
www.scholarjournals.org

JOURNAL OF PHYTOLOGY

www.journal-phytology.com

Toxicity of Heavy Metals on Germination and Seedling Growth of *Salicornia brachiata*

Anubha Sharma[1*] Iti Gontia-Mishra[2], Ashwani Kumar Srivastava[1]

[1]Amity Institute of Biotechnology, Amity University, Sector-125, Noida- 201303, U.P. India
[2]Biotechnology Centre, Jawaharlal Nehru Agricultural University, Jabalpur -482004, M.P. India

Article Info

Article History

Received : 19-05-2011
Revised : 03-08-2011
Accepted : 07-08-2011

*Corresponding Author

Tel : +91 1204392195
Fax : +91 1204392295

Email:
anuu04@gmail.com

Summary

Seed is a developmental stage that is highly protective against external stresses in the plant life cycle. In this study, we analyzed toxicity of heavy metals (Cd^{2+}, $Ni^{2+}$and As^{3+}) on seed germination and seedling growth in a halophyte *Salicornia brachiata*. Germination percentage for Ni^{2+} is lower than $Cd^{2+}$and As^{3+} metal at 200 µM concentration. Shoot length and root length decreased significantly upto 400 µM and 100 µM of Ni^{2+} and As^{3+} respectively While in case of Cd^{2+} seedlings could survive up to 300 µM concentration with a minor reduction in growth. *Salicornia brachiata* can serve as substantially important plant species for phytoremediation of heavy metals at lower concentrations in saline areas.

Key Words: Heavy metals; halophyte; germination percentage; growth parameters

Introduction

Heavy metals and metalloids are an increasing environmental problem worldwide. Agricultural soils are contaminated with heavy metals by human-induced activities and are creating severe problems [1, 2]. The main sources of contamination in agricultural soils are fertilizer impurities (Cd^{2+}), use of refuge-derived compost and sewage sludge (Cd^{2+}, Ni^{2+}) [3]. Cadmium ranks the highest in terms of damage to plant growth and human health. Moreover, its uptake and accumulation in plants poses a serious health threat to humans via the food chain [4]. Arsenic is of great concern due to extensive contamination and carcinogenicity. It is associated with many types of mineral deposits especially those which include sulfide mineralization [5]. Higher concentrations of essential or nonessential metal ions of Cd, Hg, Pb, Ag, As, are deleterious to metal-sensitive enzymes, resulting in growth inhibition and death of the organism [6]. In the past few years, work has started to uncover molecular mechanisms underlying the uptake and transport of some heavy metals [7]. However, it remains to be further investigated how these metal ions affect plant growth at different developmental stages.

Recently, the responses of plants to individual metal were extensively studied [8, 9]. However, in natural soil plant systems, often have to face multiple metal stresses, and the interactive effects of two or more elements should be studied [10]. Interactive effect of toxic heavy metals have been studied for Cd and As in *Solanum nigrum* [11], and Cd and Cr in *Dalbergia sissoo* [12] which adversely affected the growth, biomass and photosynthesis of these plants. Most of these studies have been conducted using adult plants [13, 14, 15, 16]. In a few studies, the seeds have been exposed to heavy metals [17, 18]. Seed is a stage in the plant life cycle that is well protected against various stresses. However, soon after imbibition and subsequent vegetative developmental processes, they become sensitive to stress. Germinating seeds and developing seedlings are more sensitive to metal elements than mature plants, as their defense mechanisms are not yet fully developed [19].

In highly contaminated coastal saline areas the halophyte plants can be used to phytoremediate heavy metal pollution. Mangroves highly tolerant to heavy metals are restricted to brackish water only and hence serve only a limited purpose [20]. In coastal saline areas heavy metal pollution could only be remediated by using plant species capable of growing in highly saline conditions. It would, therefore, be beneficial to explore the potential of heavy metal tolerance by various halotolerant species. Reports on the concurrent behaviour of salinity and trace elements in the soil-plant system are few. Therefore, in the present study we have selected *Salicornia brachiata* Roxburgh (Amaranthaceae) for studying effect of heavy metals. Salicornia is highly salt-tolerant and can accumulate 30-40% NaCl in its dry weight. In this study we have explored the ability of *S. brachiata* to germinate and grow in medium containing Cd^{2+}, Ni^{2+} and As^{3+} metal.

Materials and Methods

S. brachiata were collected from the salt marshes of the western coastline of Gujarat. Seeds were harvested from the dried stem of *S. brachiata* plant. During the first 2 weeks, the fresh seeds were tested for germination behavior or stored at 18ºC.

Seeds of *S. brachiata* were surface sterilized with 2% sodium hypochloride for 5 min. Seeds were then washed thoroughly 4-5 times with sterilized distilled water. Seeds were then, placed on MS basal medium [21] supplemented with 200 mM NaCl. For metal treatment MS basal media was supplemented with 50-300 µM $CdCl_2.H_2O$, 50-800 µM

$NiCl_2.6H_2O$ and 50-300 µM $NaAsO_2$ (Hi-media, India) separately for each heavy metal stress. The plates were incubated for 15 days at 25 ± 1ºC with 16 h light and 8 h dark-light cycles.

Germination was recorded every alternate day for 15 days. Germinated seeds were counted, and germination percentage, germination index and vigour index were then calculated. Each treatment consisted of 50 seeds per petri dish. Seeds with emerged radical were considered germinated. Germination percentage was calculated as germinated seeds/total seeds×100. Germination index was calculated as Gt/Dt, where GI is the germination index, Gt and Dt are the amount of the germinated seeds and the germination time, respectively. Vigour index was determined as VI = (Mean of shoot length + Mean of root length)/Germination Percentage. Growth traits were measured in terms of number of healthy plantlet, shoot length (cm), root length (cm) and fresh weight after 15 d of treatment.

Chlorophyll was estimated using the standard protocol [22]. Tissue for chlorophyll estimation was washed three times with deionized water and blotted on tissue paper. Plant tissue (0.1 g) was homogenized in 80% acetone and incubated in the dark for 6 h. The homogenate was centrifuged at 10,000 rpm for 10 min. The supernatant obtained was read at 645 nm, 665 nm in Spectra Max plus (Molecular Device, USA).

Each experiment was repeated three times and the mean values and standard deviations were then calculated. For all treatments, single factor ANOVA was carried out using Microsoft Excel followed by Dunnett's test. Dunnett's test was conducted by computing the t-test between the mean value of treatment and the control. Mean values higher than the Dunnett table value were considered significant in comparison to the control and marked accordingly.

Result and Discussion

Seed germination percentage, germination index and vigour index of *S brachiata* seeds treated with heavy metals viz. Cd^{2+}, Ni^{2+} and As^{3+} are listed in Table 1. Increasing concentrations of Cd^{2+}, Ni^{2+} and As^{3+} gradually decreased germination percentage, germination index and vigour index as compared to control seeds. When seed germination is compared for Cd^{2+}, Ni^{2+} and As^{3+} at 200 µM concentrations, germination percentage for Ni^{2+} is lower than other two metals at same concentration. Further at highest concentrations of Ni^{2+} resulted in as low as 16% of seed germinated, which was significantly lower than germination percentage at highest concentration of other metals in the study. Hence, it is evident that Ni^{2+} showed great toxicity among the all metal treatments at the time of seed germination.

Total fresh weight, shoot length and root length of the *S. brachiata* seedling were recorded for Cd^{2+} (50-300 µM), Ni^{2+} (50-800 µM) and As^{3+} (50-300 µM) metals after 15 days of treatments as shown in Table 2. Seedlings could survive up to 300 µM concentration of Cd^{2+} with a minor reduction in overall growth. The fresh weight, shoot length and root length was affected significantly at all concentrations of Cd^{2+} as compared with control. For Ni^{2+} and As^{3+} treatments, plants could tolerate 400 µM and 100 µM concentrations, respectively; however, higher concentrations these metals proven to be detrimental. Ni^{2+} shown toxic effect on seedling as the concentration of Ni^{2+} increases, seedling growth were drastically reduced. Shoot length and root length were decreased significantly upto 400 µM and 100 µM of Ni^{2+} and As^{3+} respectively. There was significant change in fresh weight for both Ni^{2+} and As^{3+} treatments. Apparently, *S. brachiata* seedling showed no symptoms of chlorosis in the 50 µM concentration of Cd^{2+} treatment. However, the chlorophyll content was decreased gradually with the increasing concentrations of all three metals treatments in comparison to control seedlings.

Table 1 Effect of heavy metals on seed germination of *Salicornia brachiata*

Metal treatment	Mean ±SE	% Germination	Germination Index	Vigour Index
Control	46.6 ±1.52	93.2%	3.11	304.45
Cd_{50}	28.0 ±1.00*	56.0%	1.86	136.26
Cd_{100}	22.3 ±1.52*	44.6%	1.48	81.91
Cd_{200}	21.6±0.57*	43.2%	1.44	73.87
Cd_{300}	15.0±1.00*	30.0%	1.00	50.1
Ni_{50}	23.6±1.15*	47.2%	1.57	97.54
Ni_{200}	16.3±0.57*	32.6%	1.08	72.48
Ni_{400}	15.6±1.15*	31.2%	1.04	35.36
Ni_{800}	8.0±1.00*	16.0%	0.53	0
As_{50}	30.6±1.52*	61.2%	2.04	78.54
As_{100}	24.3±0.57*	48.6%	1.60	43.15
As_{200}	20.6±0.57*	41.2%	1.37	0
As_{300}	13.0±1.00*	26.0%	0.86	0

*Represents the values are significantly higher/lower than control plants according to Dunnett's table

Table 2 Effect of heavy metals on growth parameters and chlorophyll content of *Salicornia brachiata*

Treatment	No. of healthy plantlet	Health	Shoot length (cm) (Mean ± S.D.)	Root length (cm) (Mean ± S.D.)	Fresh weight (g) (Mean ± S.D.)	Chlorophyll (µg.g^{-1} tissue)
Control	6/6	+++	1.93 ± 0.05	1.33 ± 0.15	0.258 ± 0.009	246.33 ± 7.02
Cd_{50}	6/6	+++	1.26 ± 0.05*	1.16 ± 0.05*	0.231 ± 0.003*	238.33 ± 3.05
Cd_{100}	6/6	+++	1.00 ± 0.22*	0.93 ± 0.14*	0.215 ± 0.004*	211.66 ± 3.05*
Cd_{200}	6/6	++	0.88 ± 0.10*	0.87 ± 0.14*	0.206 ± 0.009*	168.00 ± 4.00*
Cd_{300}	6/6	++	0.80 ± 0.05*	0.78 ± 0.11*	0.199 ± 0.009*	153.66 ± 4.04*
Ni_{50}	5/6	++	1.03 ± 0.05*	1.03 ± 0.05*	0.223 ± 0.004*	219.09 ± 4.00*
Ni_{200}	4/6	++	1.05 ± 0.17*.	1.16 ± 0.07*	0.181 ± 0.004*	198.09 ± 5.15*
Ni_{400}	3/6	+	0.54 ± 0.00*	0.63 ± 0.12*	0.154 ± 0.005*	152.00 ± 4.35*
As_{50}	3/6	++	0.53 ± 0.05*	0.75±0.25*	0.214 ± 0.009*	223.33 ± 6.11*
As_{100}	3/6	+	0.58 ± 0.08*	0.96±0.05*	0.178 ± 0.006*	175.66 ± 11.50*

Data represent mean value of three replicates and standard deviation; *represents the values are significantly higher/lower than control plants according to Dunnett's table. +++ = Good, ++ = Average, + = Poor

It is well known that germination percentage is an important indicator used to evaluate seed quality [23]. Germination index and vigour index can reflect the germination status. Here, germination percentage, germination index and vigour index were selected to evaluate the effect of Cd^{2+}, Ni^{2+} and As^{3+} on the seed germination. The study presented here showed a significant Ni-induced reduction in germination rate, fresh weight, and length of root and shoot of *S. brachiata* seedling. The results are consistent with earlier studies of various crops which have shown that higher levels of trace elements including Ni cause a significant reduction in growth parameters and other essential metabolites [24, 25]. Decrease in chlorophyll is one of the common symptoms of heavy metal toxicity in plants [26]. The reduced chlorophyll accumulation during heavy metal treatment is due to the interference of different chlorophyll biosynthesis stages.

Cd is not an essential element to plants but the contamination therewith could lead to anatomical and physiological changes [19, 27]. In this experiment seedlings could survive up to 300 µM concentration of Cd^{2+} with a minor reduction in overall growth. The fresh weight, shoot length and root length were affected significantly at all concentrations of Cd^{2+} as compared to control. Similar, results for reduction of plant fresh weight under Cd treatment was noted in *Vigna radiata* [28]. The growth inhibition produced by Cd is partially due to the effect of this heavy metal on the photosynthesis rate [29].

For As treatment, Salicornia seedlings could tolerate upto 100 µM concentration and higher concentrations of As^{3+} were detrimental for the seedlings. Shoot length and root length were decreased significantly upto 100 µM concentration of As^{3+}. It has been reported that plants can develop toxicity symptoms while they are exposed to excess arsenic in soil such as: inhibition of seed germination [30], decrease in plant height [31, 32], reduction in root growth and decrease in shoot growth [33, 34].

Conclusion

Our findings suggest that Salicornia seedlings showed a similar trend of sensitivity with increasing concentrations of Cd^{2+}, As^{3+} and Ni^{2+}. It was also noted that Ni^{2+} showed highest toxicity among the all the metal treatments at the time of seed germination. It is evident from this study that *Salicornia brachiata* can serve as an important plant species for phytoremediation of heavy metal at lower concentrations in saline areas.

Acknowledgment

The authors are thankful to Director General of Amity Institute of Biotechnology, Amity University, Noida (U.P.) India for providing financial assistance, facilities and support.

References

[1] Ali, S., F. Zeng, L. Qiu, and G. Zhang. 2011. The effect of chromium and aluminum on growth, root morphology, photosynthetic parameters and transpiration of the two barley cultivars. Biologia Plantarum 55:291-296.

[2] Ahmad, M.S.A., M. Ashraf, Q. Tabassam, M. Hussain, and H. Firdous. 2011. Lead (Pb)- induced regulation of growth, photosynthesis and mineral nutrition in maize (*Zea mays* L.) Biol. Trace Elem. Res., Doi 10.1007/s1201101190995.

[3] Alloway, B.J. 1995. Heavy Metals in Soils. Blackie Academic and Professional, Glasgow.

[4] Singh, A., C.S. Kumar and A. Agarwal. 2011. Phytotoxicity of cadmium and lead in *Hydrilla verticillata* (l.f.) Royle. J. Phytol. 3:1-4.

[5] Gani Molla M.O., M.A. Islam and M. Hasanuzzaman. 2010. Identification of arsenic hyperaccumulating plants for the development of phytomitigation technology. J. Phytol. 2: 41-48.

[6] Upadhyaya, H., S.K. Panda, M.K. Bhattacharjee and S. Dutta. 2010. Role of arbuscular mycorrhiza in heavy metal tolerance in plants: prospects for phytoremidiation. J. Phytol. 2: 16-27.

[7] Song, W.Y., E.J. Sohn, E. Martinoia, Y.J. Lee, Y.Y. Yang, M. Jasinski, C. Forestier, I. Hwang and Y. Lee. 2003. Engineering tolerance and accumulation of lead and cadmium in transgenic plants. Nat. Biotechnol. 21:914-919.

[8] Daud, M.K., M.T. Variath, S. Ali, U. Najeeb, M. Jamil, Y. Hayat, M. Dawood, M.I. Khan, M. Zaffar, S.A. Cheema, X.H. Tong and S. Zhu. 2009. Cadmium-induced ultramorphological and physiological changes in leaves of

two transgenic cotton cultivars and their wild relative. J. Hazard. Mater. 168:614-625.
[9] Nenova, V., M. Merakchiyska, G. Ganeva, E. Zozikova and S. Landjeva. 2009. Physiological responses of wheat (*Triticum aestivum* L.) - *Aegilops sharonensis* introgression lines to excess copper. J. Agron. Crop Sci. 195:197-203.
[10] An, Y.J., Y.M. Kim, T.I. Kwon and S.W. Jeong. 2004. Combined effect of copper, cadmium, and lead upon *Cucumis sativus* growth and bioaccumulation. Sci. Total Environ. 326:85-93.
[11] Sun Y.B., Q.X. Zhou and C.Y. Diao. 2008. Effects of cadmium and arsenic on growth and metal accumulation of Cd hyperaccumulator *Solanum nigrum* L. Biores. Technol. 99:1103-1110.
[12] Shah, Z.R., N. Ahmad, K.R. Masood and D.M. Zahid. 2008. The influence of cadmium and chromium on the biomass production of shisham (*Dalbergia sissoo* L.) seedlings. Pak. J. Bot. 40:1341-1348.
[13] Chatterjee, J. and C., Chatterjee. 2000. Phytotoxicity of cobalt, chromium, and copper in cauliflower. Environ. Poll. 109:69-74.
[14] Gratton, W.S., Nkongolo K.K., Spiers G.A. 2000. Heavy metal accumulation in soil and Jack pine (*Pinus banksiana*) needles in Sudbury, Ontario, Canada. Bull. Environ. Cont. Toxicol. 64:550-557.
[15] Lutts, S., I. Lefévre, C. Delpérée, S. Kivits, C. Dechamps, A. Robledo and E. Correal. 2004. Heavy metal accumulation by the halophyte species Mediterranean saltbush. J. Environ. Qual. 33:1271-1279.
[16] Sharma, A., I. Gontia, P.K. Agarwal and B. Jha. 2010. Accumulation of heavy metals and its biochemical responses in *Salicornia brachiata*, an extreme halophyte. Marine Biol. Res. 6:511-518.
[17] Claire, L.C., D.C., Adriano, K.S. Sajwan, S.L. Abel, D.P. Thoma and J.T. Driver. 1991. Effects of selected trace metals on germinating seeds of six plant species. Water, Air, Soil Poll. 59:231-240.
[18] Xiong, Z.T. 1998. Lead uptake and effects on seed germination and plant growth in a Pb hyperaccumulator *Brassica pekinensis* Rupr. Bull Environ. Cont. Toxicol. 60:285-291.
[19] Liu, X., S. Zhang, X. Shan and Y.G. Zhu. 2005. Toxicity of arsenate and arsenite on germination, seedling growth and amylolytic activity of wheat. Chemosphere 61:293-301.
[20] Macfarlane, G.R. and M.D. Burchett. 2001. Photosynthetic pigments and peroxidase activity as indicators of heavy metal stress in the grey mangrove, *Avicennia marina* (Forsk.)-Vierh. Marine Poll. Bull. 42:233-40.
[21] Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant.15:431-97.
[22] Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol. 24:1-15.
[23] Liu, T.T., P. Wu, L.H. Wang and Q. Zhou. 2011. Response of soybean seed germination to cadmium and acid rain. Biol. Trace Elem. Res. Doi. 10.1007/s1201101190536.
[24] Peralta J.R., J.L. Gardea-Torresdey, K.J. Tiemann, E. Gomes, S., Arteaga, E. Rascon and J.G. Parsons. 2001. Uptake and effects of five heavy metals on seed germination and plant growth in alfalfa (*Medicago sativa*). Bull. Environ. Cont.Toxicol. 66:727-734.
[25] Eman, A.E., N. Gad and N.M. Badran. 2007. Effect of cobalt and nickel on plant growth, yield and flavonoids content of *Hibiscus sabdariffa* L. Aus. J. Basic Appl. Sci. 1:73-78.
[26] Gajewska, E., M.,Sklodowska Slaba M., Mazur J. 2006. Effect of nickel on antioxidative enzyme activities, proline and chlorophyll content in wheat shoots. Biologia Plantarum, 50:653-59.
[27] Chaoui, A. and E.E., Ferjani. 2005. Effects of cadmium and copper on antioxidant capacities, lignification and auxin degradation in leaves of pea (*Pisum sativum* L.) seedlings. Cri. Rev. Biol. 328:23-31.
[28] Kumari, M., V. K. Sinha, A. Srivastava and V.P. Singh, 2011. Cytogenetic effects of individual and combined treatment of Cd^{2+}, Cu^{2+} and Zn^{2+} in *Vigna radiata* (L.) Wilczeck. J. Phytol. 3:38-42.
[29] Metwally, A., I. Finkermeier, M. Georgi and K.J. Dietz. 2003. Salicylic acid alleviates the cadmium toxicity in barley seedlings. Plant Physiol. 132:272-281.
[30] Liebig, Jr. G.P. 1966. Arsenic. In: Chapman HD (ed) Diagnostic Criteria for Plants and Soils. University of California, Division of Agricultural Science, Riverside, California 13-23.
[31] Marin, A.R., P.H. Masscheleyn and W.H. Patrick Jr. 1992. The influence of chemical form and concentration of arsenic on rice growth and tissue arsenic concentration. Plant Soil 139:175-183.
[32] Carbonell-Barrachina, A.A., F., Burlo-Carbonell and J. Mataix-Beneyto. 1995. Arsenic uptake, distribution, and accumulation in tomato plants: effect of arsenic on plant growth and yield. J. Plant Nutr. 18:1237-1250.
[33] Tang T. and D.M. Miller. 1991. Growth and tissue composition of rice grown in soil treated with inorganic copper, nickel, and arsenic. Commun. Soil Sci. Plant Anal. 22:2037-2045.
[34] Carbonell-Barrachina, A.A., M.A. Aarabi, R.D. DeLaune, R.P. Gambrell and W.H. Patrick Jr. 1998. The influence of arsenic chemical form and concentration on *Spartina patens* and *Partina alterniflora* growth and tissue arsenic concentration. Plant Soil 198:33-43.